Version with Markings to Show Changes Made:

In The Specification:

Please amend the paragraph on page 6, lines 6-11, to read as follows:

The invention is thus based on the identification of a novel human cyclic nucleotide phosphodiesterase. The invention encompasses a long and short form of the phosphodiesterase. The amino acid sequence of the longer form is shown in SEQ ID NO:1 [SEQ ID NO 1] and the amino acid sequence of the shorter form is shown as SEQ ID NO:3 [SEQ ID NO 3]. The nucleotide sequence of the longer form is shown as SEQ ID NO:2 or SEQ ID NO:4 [SEQ ID NO 2 or SEQ ID NO 4] and the nucleotide sequence of the shorter form is shown as SEQ ID NO:4 [SEQ ID NO 4].

Please amend the paragraph on page 6, lines 12-16, to read as follows:

The invention provides isolated phosphodiesterase polypeptides, including a polypeptide having the amino acid sequence shown in SEQ ID NO:1 or SEQ ID NO:3 [SEQ ID NO 1 or SEQ ID NO 3] or the amino acid sequence encoded by the cDNA deposited as ATCC No. PTA-1644 on April 5, 2000 ATCC. [No. _____ on _____ ("the deposited cDNA"), or as ATCC No. _____ on _____ ("the deposited cDNA").]

Please amend the paragraph on page 6, lines 17-19, to read as follows:

The invention also provides isolated phosphodiesterase nucleic acid molecules having the sequence shown in SEQ ID NO:2 or SEQ ID NO:4 [SEQ ID NO 2 or SEQ ID NO 4] or in the deposited cDNA.

Please amend the paragraph on page 6, lines 20-22, to read as follows:

The invention also provides variant polypeptides having an amino acid sequence that is substantially homologous to the amino acid sequence shown in SEQ ID NO:1 or SEQ ID NO:3

[SEQ ID NO 1 or SEQ ID NO 3] or encoded by the deposited cDNA.

Please amend the paragraph on page 6, lines 23-25, to read as follows:

The invention also provides variant nucleic acid sequences that are substantially homologous to the nucleotide sequence shown in SEQ ID NO:2 or SEQ ID NO:4 [SEQ ID NO 2 or SEQ ID NO 4] or in the deposited cDNA.

Please amend the paragraph on page 6, lines 25-27, to read as follows:

The invention also provides fragments of the polypeptide shown in SEQ ID NO:1 or SEQ ID NO:3 [SEQ ID NO 1 or SEQ ID NO 3] and nucleotide sequence shown in SEQ ID NO:2 or SEQ ID NO:4 [SEQ ID NO 2 or SEQ ID NO 4], as well as substantially homologous fragments of the polypeptide or nucleic acid.

Please amend the paragraph on page 7, lines 26-29, to read as follows:

Figure 1 shows the long phosphodiesterase nucleotide sequence (SEQ ID NO:2 [SEQ ID NO 2]) and the deduced amino acid sequence (SEQ ID NO:1 [SEQ ID NO 1]). It is predicted that amino acids 1-223 constitute the aminoterminal regulatory domain, amino acids 224-462 constitute the catalytic domain, and amino acids 463-502 constitute the carboxyterminal domain.

Please amend the paragraph on page 9, lines 4-7, to read as follows:

Figure 6 shows the short phosphodiesterase nucleotide sequence (SEQ ID NO:4 [SEQ ID NO 4]) and the deduced amino acid sequence (SEQ ID NO:3 [SEQ ID NO 3]). It is predicted that amino acids 1-223 constitute the amino terminal regulatory domain, and amino acids 224-320 constitute the catalytic domain.

Please amend the paragraph on page 11, lines 10-13, to read as follows:

The invention thus relates to a novel phosphodiesterase having the deduced amino acid sequence shown in Figure 1 or Figure 6 (SEQ ID NO:1 [SEQ ID NO 1]or SEQ ID NO:3 [SEQ ID NO 3]) or having the amino acid sequence encoded by the deposited cDNA, ATCC No. PTA-1644.

Please amend the paragraph on page 11, lines 20-24, to read as follows:

"Phosphodiesterase polypeptide" or "phosphodiesterase protein" refers to the polypeptides in SEQ ID NO:1 [SEQ ID NO 1]or SEQ ID NO:3 [SEQ ID NO 3] or encoded by the deposited cDNAs. The term "phosphodiesterase protein" or "phosphodiesterase polypeptide", however, further includes the numerous variants described herein, as well as fragments derived from the full-length phosphodiesterases and variants.

Please amend the paragraph on page 12, lines 3-5, to read as follows:

The phosphodiesterases include a catalytic signature, HDVDHPG, at residues 265-271. The sequence includes HDXXHXX (SEQ ID NO:40) [HXXDHXX], a consensus amino acid sequence in cyclic nucleotide phosphodiesterases.

Please amend the paragraph on page 13, lines 9-26, to read as follows:

In one embodiment, the phosphodiesterase polypeptide comprises the amino acid sequence shown in SEQ ID NO:1 [SEQ ID NO 1] or SEQ ID NO:3 [SEQ ID NO 3]. However, the invention also encompasses sequence variants. Variants include a substantially homologous protein encoded by the same genetic locus in an organism, i.e., an allelic variant. The phosphodiesterase has been

mapped to human chromosome 6 (6q21-q23.2), with flanking markers AFMA074ZG9 (2.6cR) and AFM214ZF6 (7.9cR). Mutations near this locus include, but are not limited to, the following: PPAC, arthropathy, progressive pseudorheumatoid, of childhood; ODDD, oculodentodigital dysplasia; heterocellular hereditary persistence of fetal hemoglobin; DFNA10, deafness, autosomal dominant nonsyndromic sensorineural 10; CMD1F, cardiomyopathy, dilated, 1F; and diabetes mellitus, transient neonatal. In the mouse this locus is associated with the following: gl, grey-lethal; dl, downless; Cat5, dominant cataract 5; Lwq3, liver weight QTL 3; mshi, male sterility and histoincompatibility; Mop2, morphine preference 2; H60, histocompatibility 60; Daq4, directional asymmetry QTL 4; Daq5, directional asymmetry QTL 5; and kd / kidney disease. Genes near this locus include PDNP1 (phosphodiesterase I/nucleotide pyrophosphatase 1 (homologous to mouseLy-41 antigen)), MACS, PTPRK, ARG1, PCMT1, DFNA10, MEKK5, CTGF, SGK, HIVEP2, CMD1F, EPB41L2, HPFH, UTRN, IFNGR1, and ESR1.

Please amend the paragraph on page 13, line 27 to page 14, line 3, to read as follows:

Variants also encompass proteins derived from other genetic loci in an organism, but having substantial homology to the phosphodiesterase of SEQ ID NO:1 [SEQ ID NO 1] or SEQ ID NO:3 [SEQ ID NO 3]. Variants also include proteins substantially homologous to the phosphodiesterase but derived from another organism, i.e., an ortholog. Variants also include proteins that are substantially homologous to the phosphodiesterase that are produced by chemical synthesis. Variants also include proteins that are substantially homologous to the phosphodiesterase that are produced by recombinant methods. It is understood, however, that variants exclude any amino acid sequences disclosed prior to the invention.

Please amend the paragraph on page 14, lines 4-10, to read as follows:

As used herein, two proteins (or a region of the proteins) are substantially homologous when the amino acid sequences are at least about 70-75%, typically at least about 80-85%, and most typically at least about 90-95% or more homologous. A substantially homologous amino acid sequence, according to the present invention, will be encoded by a nucleic acid sequence

hybridizing to the nucleic acid sequence, or portion thereof, of the sequence shown in SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4] under stringent conditions as more fully described below.

Please amend the paragraph on page 17, lines 1-8, to read as follows:

A preferred, non-limiting example of such a mathematical algorithm is described in Karlin *et al.* (1993) *Proc. Natl. Acad. Sci.* USA *90*:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs (version 2.0) as described in Altschul *et al.* (1997) *Nucleic Acids Res. 25*:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., NBLAST) can be used. See http://www.ncbi.nlm.nih.gov [http://www.ncbi.nlm.nih.gov]. In one embodiment, parameters for sequence comparison can be set at score = 100, wordlength = 12, or can be varied (e.g., W = 5 or W = 20).

Please amend the paragraph on page 17, lines 9-18, to read as follows:

In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman *et al.* (1970) (*J. Mol. Biol. 48*:444-453) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com [http://www.gcg.com]), using either a BLOSUM 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (Devereux *et al.* (1984) *Nucleic Acids Res. 12*(1):387) (available at http://www.gcg.com [http://www.gcg.com]), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6.

Please amend the paragraph on page 19, lines 8-11, to read as follows:

The invention thus also includes polypeptide fragments of the phosphodiesterase. Fragments can be derived from the amino acid sequence shown in SEQ ID NO:1 [SEQ ID NO 1] or SEQ ID NO:3 [SEQ ID NO 3]. However, the invention also encompasses fragments of the variants of the

phosphodiesterases as described herein.

Please amend the paragraph on page 25, lines 4-16, to read as follows:

The protein sequences of the present invention can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to the nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the proteins of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov [http://www.ncbi.nlm.nih.gov].

Please amend the paragraph on page 38, lines 20-24, to read as follows:

The nucleotide sequences in SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4] were obtained by sequencing the deposited human cDNA. Accordingly, the sequence of the deposited clone is controlling as to any discrepancies between the two and any reference to the sequences of SEQ ID NO 2 [SEQ ID NO 2] or SEQ ID NO 4 [SEQ ID NO 4] includes reference to the sequences of the deposited cDNA.

Please amend the paragraph on page 38, lines 25-26, to read as follows:

The specifically disclosed cDNAs comprise the coding region and 5' and 3' untranslated sequences in SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4].

Please amend the paragraph on page 39, lines 1-6, to read as follows:

The invention provides isolated polynucleotides encoding the novel phosphodiesterases. The term "phosphodiesterase polynucleotide" or "phosphodiesterase nucleic acid" refers to the sequences shown in SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4] or in the deposited cDNAs. The term "phosphodiesterase polynucleotide" or "phosphodiesterase nucleic acid" further includes variants and fragments of the phosphodiesterase polynucleotides.

Please amend the paragraph on page 41, lines 1-3, to read as follows:

Phosphodiesterase nucleic acid can comprise the nucleotide sequences shown in SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4], corresponding to human osteoblast (short form) and kidney and adrenal gland (long form) cDNA.

Please amend the paragraph on page 41, lines 6-9, to read as follows:

The invention further provides variant phosphodiesterase polynucleotides, and fragments thereof, that differ from the nucleotide sequences shown in SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4] due to degeneracy of the genetic code and thus encode the same protein as that encoded by the nucleotide sequences shown in SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4].

Please amend the paragraph on page 41, lines 18-21, to read as follows:

Typically, variants have a substantial identity with a nucleic acid molecules of SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4] and the complements thereof. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions.

Please amend the paragraph on page 41, line 22 to page 42, line 2, to read as follows:

Orthologs, homologs, and allelic variants can be identified using methods well known in the art. These variants comprise a nucleotide sequence encoding a phosphodiesterase that is at least about 60-65%, 65-70%, typically at least about 70-75%, more typically at least about 80-85%, and

most typically at least about 90-95% or more homologous to the nucleotide sequence shown in SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4] or a fragment of this sequence. Such nucleic acid molecules can readily be identified as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4] or a fragment of the sequence. It is understood that stringent hybridization does not indicate substantial homology where it is due to general homology, such as poly A sequences, or sequences common to all or most proteins, all cyclic nucleotide phosphodiesterases, or all Family 7 phosphodiesterases. Moreover, it is understood that variants do not include any of the nucleic acid sequences that may have been disclosed prior to the invention.

Please amend the paragraph on page 42, lines 3-23, to read as follows:

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences encoding a polypeptide at least about 60-65% homologous to each other typically remain hybridized to each other. The conditions can be such that sequences at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 95% or more identical to each other remain hybridized to one another. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6, incorporated by reference. One example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. In another non-limiting example, nucleic acid molecules are allowed to hybridize in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more low stringency washes in 0.2X SSC/0.1% SDS at room temperature, or by one or more moderate stringency washes in 0.2X SSC/0.1% SDS at 42°C, or washed in 0.2X SSC/0.1% SDS at 65°C for high stringency. In one embodiment, an isolated nucleic acid molecule that hybridizes under stringent conditions to the sequence of SEQ ID NO:1 [SEQ ID NO 1] or SEQ ID NO:3 [SEQ ID NO 3] corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-

occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

Please amend the paragraph on page 42, line 32 to page 43, line 9, to read as follows:

The present invention also provides isolated nucleic acids that contain a single or double stranded fragment or portion that hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4] or the complement of SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4]. In one embodiment, the nucleic acid consists of a portion of the nucleotide sequence of SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4] and the complement of SEQ ID NO:2 [SEQ ID NO 4] or SEQ ID NO:4 [SEQ ID NO 4]. The nucleic acid fragments of the invention are at least about 15, preferably at least about 18, 20, 23 or 25 nucleotides, and can be 30, 40, 50, 100, 200, 500 or more nucleotides in length. Longer fragments, for example, 30 or more nucleotides in length, which encode antigenic proteins or polypeptides described herein are useful.

Please amend the paragraph on page 44, lines 10-20, to read as follows:

The nucleotide sequences of the present invention can be used as a "query sequence" to perform a search against public databases, for example, to identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul *et al.* (1990) *J. Mol. Biol. 215*:403-10. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to the proteins of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res. 25(17)*:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov [http://www.ncbi.nlm.nih.gov].

Please amend the paragraph on page 44, lines 21-30, to read as follows:

The nucleic acid fragments of the invention provide probes or primers in assays such as those described below. "Probes" are oligonucleotides that hybridize in a base-specific manner to a complementary strand of nucleic acid. Such probes include polypeptide nucleic acids, as described in Nielsen *et al.* (1991) *Science 254*:1497-1500. Typically, a probe comprises a region of nucleotide sequence that hybridizes under highly stringent conditions to at least about 15, typically about 20-25, and more typically about 40, 50 or 75 consecutive nucleotides of the nucleic acid sequence shown in SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4] and the complements thereof. More typically, the probe further comprises a label, e.g., radioisotope, fluorescent compound, enzyme, or enzyme co-factor.

Please amend the paragraph on page 45, lines 19-28, to read as follows:

The phosphodiesterase polynucleotides are useful as a hybridization probe for cDNA and genomic DNA to isolate a full-length cDNA and genomic clones encoding the polypeptides described in SEQ ID NO:1 [SEQ ID NO 1] or SEQ ID NO:3 [SEQ ID NO 3] and to isolate cDNA and genomic clones that correspond to variants producing the same polypeptides shown in SEQ ID NO:1 [SEQ ID NO 1] or SEQ ID NO:3 [SEQ ID NO 3] or the other variants described herein. Variants can be isolated from the same tissue and organism from which the polypeptides shown in SEQ ID NO:1 [SEQ ID NO 1] or SEQ ID NO:3 [SEQ ID NO 3] were isolated, different tissues from the same organism, or from different organisms. This method is useful for isolating genes and cDNA that are developmentally-controlled and therefore may be expressed in the same tissue or different tissues at different points in the development of an organism.

Please amend the paragraph on page 46, lines 4-7, to read as follows:

The nucleic acid probe can be, for example, the full-length cDNA of SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4], or a fragment thereof, such as an oligonucleotide of at least 12, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to mRNA or DNA.

Please amend the paragraph on page 46, line 14 to page 47, line 6, to read as follows:

Antisense nucleic acids of the invention can be designed using the nucleotide sequences of SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4], and constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest).

Please amend the paragraph on page 60, lines 1-5, to read as follows:

Examples of antisense molecules useful to inhibit nucleic acid expression include antisense molecules complementary to a fragment of the 5' untranslated region of SEQ ID NO:2 [SEQ ID NO

2] or SEQ ID NO:4 [SEQ ID NO 4] which also includes the start codon and antisense molecules which are complementary to a fragment of the 3' untranslated region of SEQ ID NO:2 [SEQ ID NO 2] or SEQ ID NO:4 [SEQ ID NO 4].